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Ultrastructural and Cytochemical Evaluation of the Cytotoxicities  
of Trimethylpentane on Rat Renal and Hepatic Tissues

Forecast and Progress Report  
William N. Norton, Ph.D.

①  
The primary objective of the present investigation is to determine the acute cytopathological effects of trimethylpentane on the hepatic and renal tissues of the sexually mature male rat. Specifically, the first year of the project is designed to evaluate, cytochemically, the activity of lysosomes associated with kidney cells of the proximal convoluted tubule, to determine by means of ferritin tracers whether the glomerular basement membrane has been compromised, and to analyze by scanning electron microscopy various regions of the kidney for manifestations of cellular toxicity. (KR) ←

Subsequent to a consultation with scientists from Wright-Patterson AFB who are knowledgeable in the area of hydrocarbon toxicity the protocol described in the submitted grant proposal was modified in 2 significant ways. The originally stated dose of 10 ml/kg body weight was considered to be too high for a critical evaluation of the initial subtle cellular changes associated with lesion development. Thus, a lower concentration of 1.5 ml/kg body weight was chosen. Additionally, the administration of trimethylpentane was altered from a single dose to exposure periods of twice weekly for 7, 14 or 28 days. The general consensus was that low consistent exposures of the experimental animals to the hydrocarbon would be more conducive to an analysis of the initial phases of lesion development. As a result of the modifications, sexually mature male rats were administered trimethylpentane by the gavage method twice weekly at a concentration of 1.5 ml/kg body weight. Control animals were given a comparable concentration of distilled water. Experimental and control rats were sacrificed at 7, 14 or 28 days subsequent to the initial exposure. All tissues to be examined during the investigation were processed at this time.

This progress report consists of a brief description of the major structural changes detected by a scanning electron microscopy analysis of control and experimental kidneys. All tissues projected for observation by scanning electron microscopy were fixed chemically by means of perfusion via the heart with a 4% solution of glutaraldehyde which was buffered with 0.1M sodium cacodylate (pH 7.4). The tissues were post-fixed with osmium tetroxide, dehydrated in a graded series of ethanol and cryofractured by submerging the material in liquid nitrogen and fracturing the latter with a chilled razor blade. The fractured tissue was critical point dried, sputter-coated with a layer of gold (90Å) and viewed on either an ETECH Autoscan or AMRAY 1200B scanning electron microscope.

The following descriptions represent a general preliminary analysis. Critical statistical data have not yet been computed. The glomeruli, proximal convoluted tubules and corticomedullary junctions represent the 3 general zones of the kidney which were investigated by scanning electron microscopy. Within the region of the glomeruli 2 structural aberrations were noted. Initial observations indicated a proliferation of microprojections associated with the primary, secondary and tertiary branches which extended from the nucleated portion of the podocyte cell body (Figs. 1,2). Such an increase of tubular extensions would be



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very difficult to detect with conventional transmission electron microscopy. Preliminary observations also indicated a significant increase in the number of membranous blebs which protruded from the secondary and tertiary branches.

An analysis of freeze-fractured proximal convoluted tubules revealed distinct regions along the inner lumen where a loss of cilia was evident (Figs. 3,4). At several sites the reduction of cilia appeared to have occurred concomitant with cellular degradation. The latter was manifested by an extrusion of cellular components into the tubular lumen. A general lysis of the plasma membrane was not evident. Cellular necrosis was noted along the length of the proximal tubule. Within the epithelial cells comprising the tubule hyaline droplets of various dimensions form as a result of exposure to the hydrocarbon. The lumina of several tubules at the corticomedullary junction contained substantial amounts of what appeared to be cellular debris.

The results obtained thus far indicate that trimethylpentane is capable of inducing consistent cellular lesions throughout the specific time points selected for observation. Several of the cellular alterations are similar to those reported for other tissues exposed to specific aromatic hydrocarbons such as benzene and toluene. However, certain structural abnormalities detected in the present study have not yet been reported for hydrocarbon cytotoxicity.

The remainder of the first year will be devoted to analyzing the cytochemical phase of the project. In addition, a statistical analysis will be conducted on the information obtained from the scanning electron micrographs. The second year of the study will be concerned with an investigation of the developmental phases of the cellular lesions associated with the kidney, and a morphometric analysis (quantification) of the endoplasmic reticulum system of hepatocytes. Transmission electron microscopy related techniques will be employed during this phase.

There is a need to acquire basic biochemical and cytological information regarding cellular responses to trimethylpentane since the hydrocarbon is capable of inducing the formation of renal tumors. The multi-facet analysis of this project should provide pertinent information concerning initial and progressive cellular changes which result from acute exposure to a neoplastic agent such as trimethylpentane.

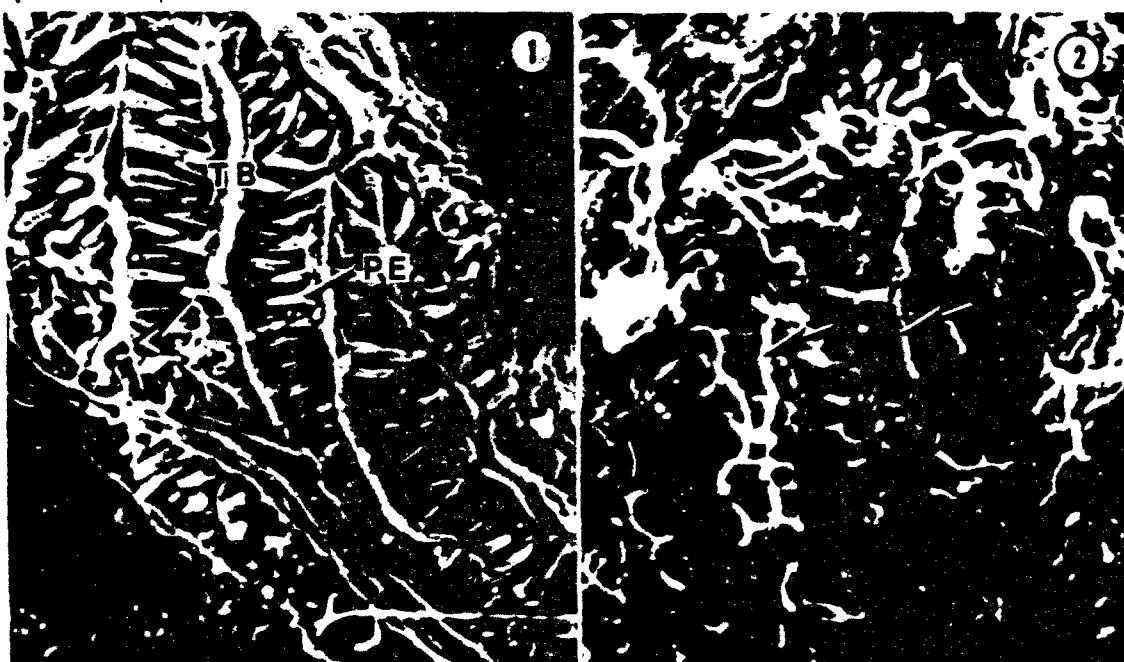


Figure 1. Scanning electron micrograph depicts a region of the glomerulus with orderly arranged pedicels (PE) and tertiary branches (TB) of podocytes. Note sparsity of microprojections (arrow). Control tissue. X 10,000.

Figure 2. Microprojections (arrows) are evident in high concentrations along the length of the glomerular capillary. Experimental tissue - 14 days of exposure. X 10,000.

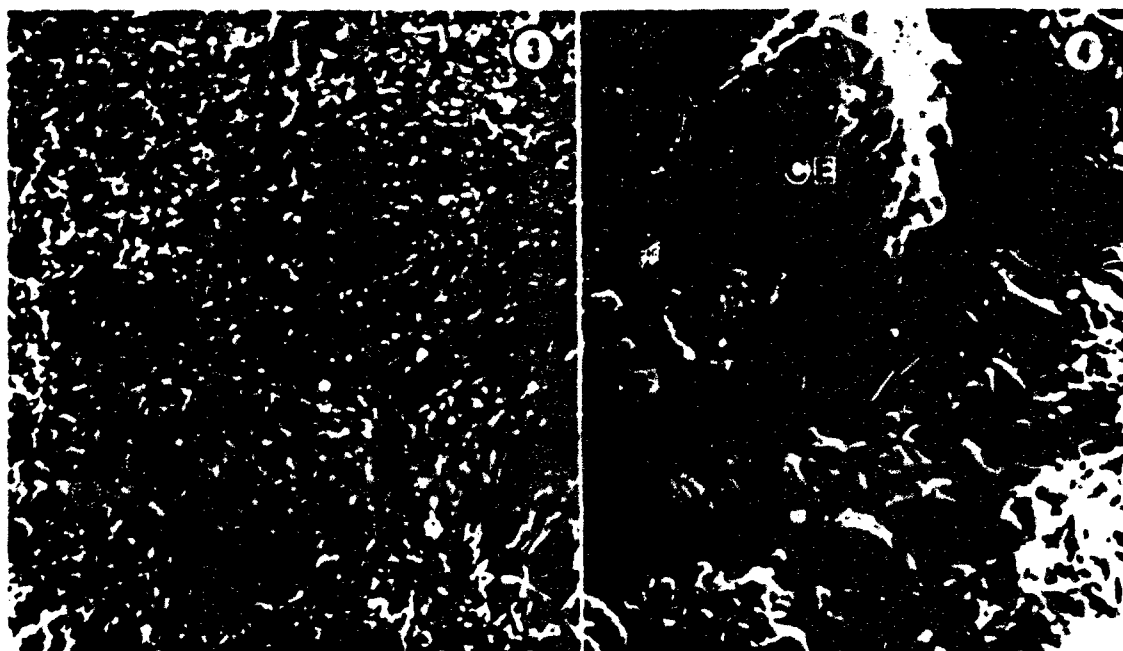


Figure 3. Compact arrays of cilia are evident along the lumen of the proximal convoluted tubule. Control tissue. X 10,000.

Figure 4. Regions of cilia reduction (arrow) occur at various sites along the proximal convoluted tubule. Note the presence of a necrotic cell (CE) within the lumen. Experimental tissue - 28 days of exposure. X 10,000.